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Grazers increase the sensitivity of coralline algae to ocean acidification and warming

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ABSTRACT

Coralline algae are expected to be adversely impacted by ocean acidification and warming. Most research on these algae has involved experiments on isolated species, without considering species interactions, such as grazing. This myopic view is challenging because the impact of climate change on coralline algae will depend on the direct impacts on individual coralline species and the indirect effects of altered interactions with other species. Here, we tested the influence of grazing on the response of the coralline alga *Lithothamnion corallioides* to near-future ocean acidification and warming. Two three-month experiments were performed in the winter and summer seasons in mesocosms under crossed conditions of pCO_2 (ambient and high pCO_2) and temperature (ambient and +3 °C) in the presence and absence of grazers. In the winter, *L. corallioides* photosynthesis decreased with rising temperature in the presence of grazers, while calcification increased. It is likely that increased calcification may act as a structural protection to prevent damage from grazing. However, increasing calcification rates in the presence of grazers may be detrimental to other physiological processes, such as photosynthesis. In the summer, *L. corallioides* primary production, respiration, and calcification were higher in the presence of grazers than in their absence. Light calcification rates were reduced under high pCO_2 in the presence of grazers only. Moreover, dark calcification rates were more adversely affected by pCO_2 increase in the presence of grazers. Through their feeding activity, grazers may alter the structural integrity of thalli and increase the sensitivity of coralline algae to ocean acidification. Our results indicate that both season and grazing play a key role in the response of *L. corallioides* to acidification and warming. Seasonal variations and species interactions are thus critical to consider to make ecologically relevant predictions of the effects of future environmental changes.

1. Introduction

Over the past 250 years, oceans have absorbed approximately one-third of atmospheric carbon dioxide (CO_2), reducing the surface water pH of 0.1 units and inducing significant changes in the water carbonate chemistry (Sabine et al., 2004). The oceans have also assimilated an important part of Earth's additional heat, increasing sea surface temperature about 0.7 °C over the last 100 years (Gattuso et al., 2015). The present atmospheric CO_2 level of 400 ppm is likely to reach 936 ppm by the end of the 21st century, under the "business as usual" scenario (RCP 8.5) (Pörtner et al., 2014). This phenomenon could lead to an ocean pH decrease of 0.33 units and a sea surface temperature increase of 2.7 °C by the end of this century (Bopp et al., 2013).

Since the last decade, the scientific community's interest in understanding how the projected ocean acidification and warming will impact marine organisms has greatly increased (Kroeker et al., 2013; Riebesell and Gattuso, 2015; Yang et al., 2016), with a specific interest regarding marine calcifiers. Despite recent studies showed the ability of some calcareous species to cope with ocean acidification (Leung et al., 2017; DeCarlo et al., 2018), most of them are considered vulnerable (Doney et al., 2009). Among calcifying species, red calcareous coralline algae (Corallinaceae, Rhodophyta) are expected to be adversely impacted by global warming and ocean acidification. They are thought to be among organisms the most vulnerable to ocean acidification due to the high solubility of their magnesium calcite skeleton (Andersson et al., 2008; Haese et al., 2014).

To date, most research has focused on the response of coralline algae at the species scale under the influence of ocean acidification and warming (Martin and Gattuso, 2009; Martin et al., 2013; Noisette et al., 2013; Hofmann and Bischof, 2014) and studies examining the species interactions in a context of global change remain still poorly documented

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(Legrand et al., 2017). Species interactions are a key element of ecosystems functioning and are likely to play an important role in the response of species in a context of climate change (O'Connor et al., 2011; Hansson et al., 2012; Kroeker et al., 2012). Several studies have documented the importance of grazing in the control of algal biomass (Sousa et al., 1981; Cloern, 2001; Guillou et al., 2002) or the stimulation of their productivity (Littler et al., 1995; Cerda et al., 2009). Despite this, the influence of grazing on the physiological response of macroalgae to climate change remains poorly understood. We hypothesized here that the presence of grazer may act as an additional pressure on maerl and increase its vulnerability to ocean acidification and warming.

The study of Legrand et al. (2017) tested the response of assemblages of free-living coralline algae *Lithothamnion corallioides*, epiphytic fleshy algae, and main grazer species (gastropods and sea urchins) under crossed conditions of pCO_2 (ambient and high pCO_2) and temperature (ambient and +3 °C). This research suggested that grazers may have both indirect (regulation of *L. corallioides* epiphytic competitors) and direct (effect on *L. corallioides* structural integrity) impacts on *L. corallioides* physiology, driving its response to climate change (Legrand et al., 2017). However, these results did not allow us to understand the specific influence of grazing on the response of coralline algae to global change. Therefore, we investigated here the physiological response of the coralline alga *L. corallioides*, to ocean acidification and warming in the presence and the absence of grazer. The results in the presence of grazers were presented in Legrand et al. (2017). As the response of species to climate change is also known to vary depending on seasonal changes in environmental factors (Godbold and Solan, 2013), we examined the impact of grazers on *L. corallioides* physiology both in winter and summer conditions.

2. Materials and methods

2.1. Biological material

Thalli of the maerl species *L. corallioides* Crouan and Crouan, 1867 were collected using a naturalist dredge (width: 1 m, height: 0.2 m, net: 1.5 m long) from a maerl bed from the bay of Brest, France (Anse du Roz, 48°19′56 N 04°19′56 W). Thalli of *L. corallioides* without any apparent epiphyte were selected. Thalli were not cleaned in order to keep epiphytes spores that could be present on their surface. The three main species of grazers living in maerl beds were also sampled: the two gastropods species *Gibbula magus* Linnaeus, 1758 and *Jujubinus exasperatus* Pennant, 1777 and the urchin species *Psammechinus miliaris* Müller, 1771 (Grall et al., 2006). For each species, only medium sized individuals were selected (Table 1). Samples were collected on January 24, 2015 (winter conditions) and September 15, 2015 (summer conditions). At each season, 1 kg of living maerl, 500 g of dead thalli of *L. corallioides,* 40 individuals of *G. magus*, 40 individuals of *P. miliaris*, and 80 individuals of *J. exasperatus*, were randomly selected and transported in seawater tanks to the Roscoff Marine Station.

2.2. Experimental set-up

The experimental design was that described in Legrand et al. (2017). Two three-month long experiments were conducted in winter (March to June 2015) and summer (September to December 2015) conditions. 20 artificial assemblages were created and randomly assigned to 20 15-L aquaria, as described in Legrand et al. (2017). Each assemblage was composed of 45 g of living *L. corallioides*, two *G. magus* individuals, two *P. miliaris* individuals and four *J. exasperatus* individuals. In addition, 20 g of living *L. corallioides* were added in each aquarium in a part separated from the grazers by a grid. The living maerl density in the parts of the aquarium with and without grazers was similar, corresponding to about 1 kg m^{-2}. In order to have more realistic assemblages, dead thalli of *L. corallioides* were also added in both parts (20 g and 9 g, respectively; about 400 g m^{-2}). This ratio is consistent with natural ratio (Hily et al., 1992). Algae and grazers were then acclimated to the laboratory conditions for 7 days. After the acclimation period, pH was gradually decreased by 0.05 units per day over 7 days through CO_2 bubbling, until reaching required experimental values. Similarly, temperature was increased by 0.5 °C per day. At each season, two pCO_2 conditions were tested and crossed with two temperature conditions to examine the interactive effect of pCO_2 and temperature. Therefore, four crossed conditions were tested:

1) ambient pCO_2 and ambient temperature (control, A-pCO_2; T).
2) high pCO_2 and ambient temperature (H-pCO_2; T).
3) ambient pCO_2 and high temperature (A-pCO_2; T + 3 °C).
4) high pCO_2 and high temperature (H-pCO_2; T + 3 °C).

Ambient pCO_2 conditions (A-pCO_2) were determined according to *in situ* winter (7.98) and summer (8.06) mean pH_T (pH on the total scale) monitored above maerl beds in the Bay of Brest (from Martin, unpublished data). Elevated pCO_2 (H-pCO_2) corresponded to the "business-as-usual" scenario predicted for the end of the century, with a pH decrease of −0.33 units (Bopp et al., 2013). Ambient temperature (T) corresponded to *in situ* winter (10.0 °C) and summer (17.1 °C) conditions in the Bay of Brest recorded by SOMLIT (from 2003 to 2014), and high temperature (T + 3 °C) was determined according to the "business-as-usual" scenario predicted for 2100 (Bopp et al., 2013). The temperature and the pH were controlled by an offline feedback system (IKS Aquastar, Karlsbad, Germany) using heaters and CO_2 bubbling, respectively (Legrand et al., 2017). The regulation was made in four 100 L header tanks, continuously supplied with filtered (5 µm) natural seawater (waterflow rate of 150 L h^{-1}). Monitoring of seawater parameters within aquaria were described in Legrand et al. (2017).

Irradiance was set to the mean *in situ* daily irradiance at 5 m depth in the Bay of Brest according to Martin et al. (2006). It was 30–40 µmol photons m^{-2} s^{-1} in winter and 90–100 µmol photons m^{-2} s^{-1} in summer. The light was provided by two or four 80 W fluorescent tubes (JBL Solar Ultra Marin Day, JBL Aquaria, Nelson, New Zealand) above the aquaria under a 10/14 h or 14/10 h light/dark photoperiod, for winter or summer conditions, respectively.

2.3. Metabolic measurements

After each three-months experiment, *L. corallioides* was cleaned of epiphytes. Physiological measurements were performed through incubations in 185 mL acrylic respirometry chambers (Engineering & Design Plastics Ltd., Cambridge, UK), both in the parts with grazers and without grazers. Incubations were carried out in the light and in the

Table 1
Mean sizes (± 1 standard deviation) of the two gastropods species *Gibbula magus* (shell diameter, *n* = 40) and *Jujubinus exasperatus* (shell height, n = 80) and the urchin species *Psammechinus miliaris* (test diameter, n = 40) maintained during the two three-month experiments conducted in winter (March to June 2015) and summer (September to December 2015) conditions.

Species		Winter experiment	Summer experiment
Gibbula magus	Shell diameter (cm)	2.51 (± 0.19 cm)	2.07 (± 0.22 cm)
Jujubinus exasperatus	Shell height (cm)	0.81 (± 0.13 cm)	0.93 (± 0.15 cm)
Psammechinus miliaris	Test diameter (cm)	1.42 (± 0.23 cm)	2.00 (± 0.15 cm)

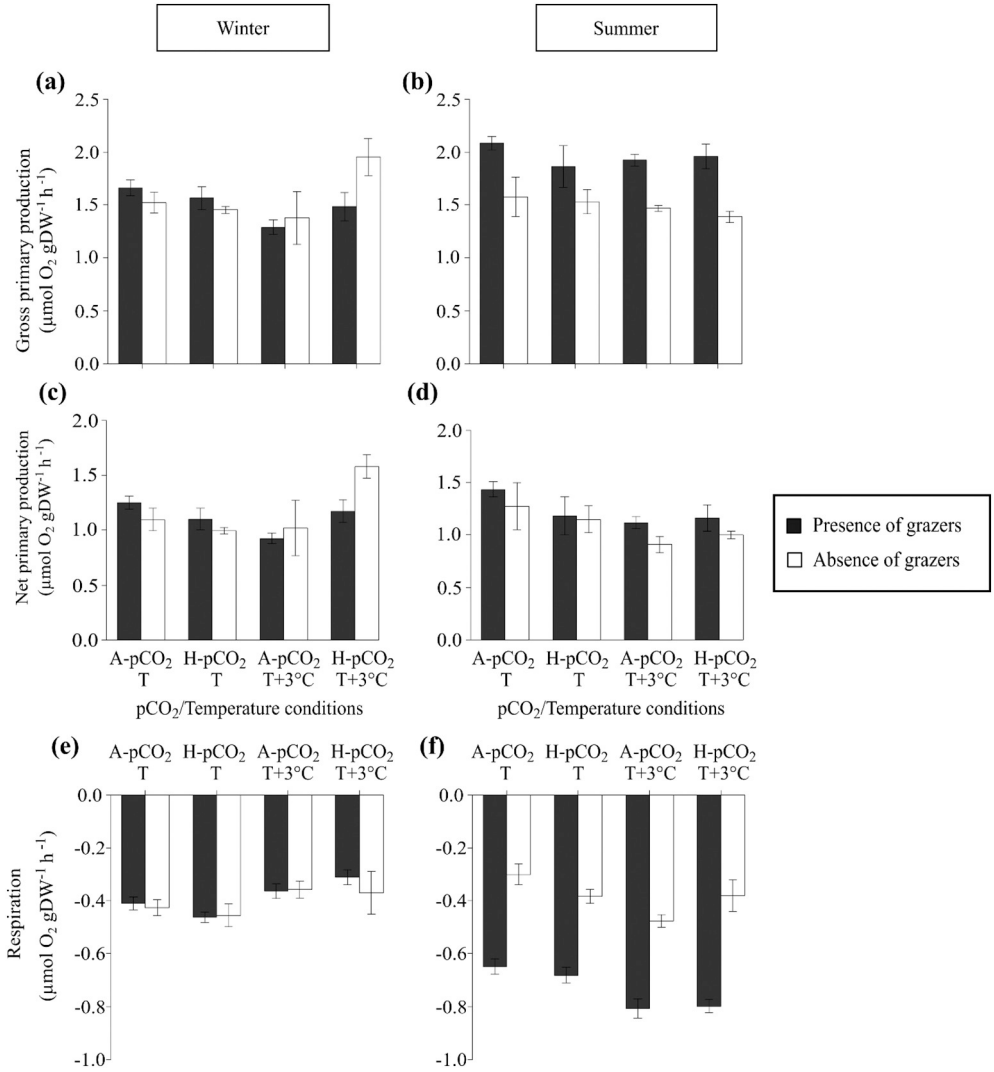

Fig. 1. Winter and summer gross (a and b, respectively) and net (c and d, respectively) primary production and respiration (e and f, respectively) of *L. corallioides* in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments, after being maintained three months in the presence (black) and absence (white) of grazers. Results are presented as means ± SE (n = 5). Results "with grazers" come from the work of Legrand et al. (2017).

dark under constant temperature. 10 to 15 g of *L. corallioides* were placed on a plastic grid above a stirring bar. The stirring bar in the chambers ensured the seawater was well mixed. Incubations were conducted during the day and lasted from 1 to 2 h according to the season and the light or dark conditions.

Net primary production (light incubations) and respiration rates (dark incubations) were calculated by measuring oxygen concentration at the beginning and at the end of incubations. Oxygen concentration measurements were carried out using a non-invasive optical fiber system (FIBOX 3, PreSens, Regensburg, Germany). Reactive spots were calibrated with 0% and 100% buffer solutions. The 0% buffer solution was prepared by dissolving 1 g of sodium sulfite (Na_2SO_3) in 100 mL of seawater. The 100% buffer solution was prepared by bubbling air into 100 mL of seawater using an air-pump for 20 min to obtain air-saturated seawater. Net primary production (NPP, in µmol O_2 gDW^{-1} h^{-1}, Eq. (1)), respiration (R, in µmol O_2 gDW^{-1} h^{-1}, Eq. (1)) and gross primary production (GPP, in µmol O_2 gDW^{-1} h^{-1}, Eq. (2)) rates were calculated as:

$$\text{NPP or R} = \frac{\Delta O_2 \times V}{\Delta t \times DW} \quad (1)$$

$$\text{GPP} = \text{NPP–R} \quad (2)$$

where ΔO_2 is the difference between the initial and final oxygen concentrations (µmol O_2 L^{-1}), V the volume of the chamber (L), Δt the incubation time (h), and DW the dry weight of the organisms incubated (g). The dry weight was obtained after 48 h at 60 °C.

Control incubations containing only seawater were carried out to correct oxygen fluxes from any biological activity in seawater. Oxygen fluxes calculated in control chambers were subtracted from oxygen fluxes of chambers containing algae.

Seawater samples were taken in each aquarium at the beginning of incubations and in the chambers at the end of incubations for measurements of total alkalinity (A_T) and ammonium (NH_4^+), as described in Legrand et al. (2017). A_T was obtained from the method of Dickson et al. (2007), using open-cell titration (automatic titrator; TitroLine alpha, Schott SI Analytics, Mainz, Germany). NH_4^+ concentrations were determined using spectrophotometry (630 nm; spectrophotometer UV-1201V, Shimadzu Corp, Kyoto, Japan) according to the Solorzano method (Solorzano, 1969). Light and dark calcification rates (G_l or G_d, in µmol $CaCO_3$ gDW^{-1} h^{-1}, Eq. (3)) were calculated using the alkalinity anomaly technique (Smith and Key, 1975) and corrected from NH_4^+ fluxes (Gazeau et al., 2015) as:

$$G_l \text{ or } G_d = \frac{(-\Delta A_T + \Delta NH_4^+) \times V}{2 \times \Delta t \times DW} \quad (3)$$

where ΔAT is the difference between the initial and final total alkalinity concentrations (μeq L^{-1}) and ΔNH_4^+ is the difference between the initial and final ammonia concentrations.

2.4. Chlorophyll a analysis

At the end of the experiments, chlorophyll *a* content was measured in *L. corallioides* thalli collected in each aquarium. Samples were immediately frozen at −20 °C pending analyses. Then samples were freeze-dried and crushed into powder using a mortar, in the dark. An aliquot of 0.15 g of powder was precisely weighed and suspended in 10 mL of 90% acetone and stored in the dark at 4 °C for 12 h. Samples were then centrifuged at 4000 rpm. The supernatant was collected, and absorbance was measured at 630, 647, 664, and 691 nm. Chlorophyll *a* concentration (μg gDW^{-1}) was calculated from Ritchie (2008).

2.5. Data analysis

Statistical analyses were carried out using the free software R 3.2.2 version (©The R Foundation for Statistical Computing). The effect of grazing, temperature and pCO_2 on the net and gross primary production, respiration, light and dark calcification and chlorophyll *a* content of *L. corallioides* was tested for each season, using three-way ANOVAs.

3. Results

In the summer, gross primary production rates (GPP) were significantly higher in the presence of grazers (+33%), while no effect of grazing was detected in the winter (Fig. 1a, b; Table 2). In the winter, *L. corallioides* net primary production (NPP) and GPP are significantly affected by the interaction between grazing and temperature and the interaction between pCO_2 and temperature (Fig. 1a, c; Table 2; Supplementary material S1a,c). In the summer, increased temperature reduced NPP (Fig. 1d; Table 2).

The presence of grazers significantly increased respiration rates (R) in the summer (+88%), while no effect was observed in the winter (Fig. 1e, f; Table 2). *L. corallioides* R was reduced under elevated temperature in the winter (Table 2). In the summer, an antagonistic effect of pCO_2 and temperature was detected on R (Table 2; Supplementary material S2a).

In the summer, an interaction of grazing and temperature was detected, with an increase in chlorophyll *a* content observed under elevated temperature in the presence of grazers, while a decrease occurred in their absence (Fig. 2; Table 2; Supplementary material S2b).

The presence of grazer increased the light calcification rates (G_l) in the winter (+50%; Fig. 3a) and this positive effect was amplified in the summer (+106%; Fig. 3b). In winter conditions, *L. corallioides* G_l was significantly higher under elevated temperature (Table 2). In the summer, an interactive effect of grazing and pCO_2 was detected on G_l, with an increase in G_l observed under high pCO_2 in the absence of grazer, while a decrease was evidenced in their presence (Table 2; Supplementary material S2c).

Grazing alone increased dark calcification rates (G_d) in the summer (+41%; Fig. 3c, d). In the winter, the positive effect of increased temperature on G_d was more pronounced in the presence of grazers (Table 2; Supplementary material S1e). Furthermore, the decline in G_d under high pCO_2 was more important in the presence of grazers (Table 2; Supplementary material S1f). In the summer, increased pCO_2 alone significantly decreased G_d (Table 2). Grazing and temperature have an interactive effect on G_d in the summer, as the decline in G_d was mainly observed under elevated temperature in the absence of grazers.

4. Discussion

The present findings revealed that the response of *L. corallioides* to ocean acidification and warming is highly related to changes in seasons and grazing conditions. Within natural ecosystems, grazers are often associated with an important ecological function, exerting a control on the composition and abundance of plant biomass (Hairston et al., 1960; Guillou et al., 2002; Bonaviri et al., 2011). In the present study, weak effects of grazing were detected on gross primary production, respiration, chlorophyll *a* content, and dark calcification of *L. corallioides* in the winter. In their study, Legrand et al. (2017) evidenced a lower metabolic rate and feeding activity of the gastropod *G. magus* and the urchin *P. miliaris* in winter, compared with summer conditions. The reduced activity of these species may decrease the grazing pressure on *L. corallioides* and thus the impact on its physiology. Moreover, the reduced metabolism of *L. corallioides* in the winter may also partly explain the relative lack of significant effect of grazing in this condition. Conversely in the summer, changes in gross primary production, respiration, chlorophyll *a* content, and calcification appeared mainly related to grazing. Positive grazer-algal interaction has often been evidenced between grazers and coralline algae (Steneck, 1982, 1983; Littler et al., 1995; Wai and Williams, 2005), grazing acting as a "service" for algae by removing algal competitors (Steneck, 1982; Carpenter, 1986; Littler et al., 1995; Wai and Williams, 2005). Grazers may also remove a part of non-photosynthetic tissues and senescent cells present on the thallus

Table 2
Analysis of variance results for the effects of grazing (presence/absence), temperature (T; ambient/elevated) and pCO_2 (ambient/elevated) on gross and net primary production, respiration, chlorophyll *a* content, and light and dark calcification rates of *L. corallioides* in the winter and summer. Statistical analyses were performed using 3-way crossed ANOVAs. Significant p-values are shown in bold (α = 0.05). Degrees of freedom = 1.

	Gross production GPP		Net production NPP		Respiration R		Chlorophyll a		Light calcification G_l		Dark calcification G_d	
Winter	F	p	F	p	F	p	F	p	F	p	F	p
Grazing	0.7	0.42	0.5	0.47	0.3	0.59	1.5	0.23	19.0	**< 0.001** ↗	0.4	0.52
T	0.1	0.80	0.6	0.45	9.5	**0.004** ↘	0.7	0.41	10.6	**0.003** ↗	9.0	**0.005**
pCO_2	2.5	0.12	2.8	0.11	0.1	0.72	0.7	0.42	3.3	0.077	100.7	**< 0.001**
Grazing × T	4.6	**0.041**	5.2	**0.030**	0.1	0.70	3.6	0.067	0.3	0.59	5.4	**0.027**
Grazing × pCO_2	1.2	0.29	1.2	0.28	0.1	0.71	0.3	0.61	0.2	0.66	14.2	**< 0.001**
pCO_2 × T	6.1	**0.019**	9.9	**0.004**	1.1	0.29	0.4	0.55	0.5	0.49	0.0	0.95
Grazing × T × pCO_2	0.9	0.36	0.6	0.43	0.6	0.45	0.0	0.91	1.9	0.18	4.1	0.052
Summer	F	p	F	p	F	p	F	p	F	p	F	p
Grazing	27.2	**< 0.001** ↗	2.6	0.12	179.6	**< 0.001** ↗	5.0	**0.033**	77.1	**< 0.001**	157.4	**< 0.001**
T	0.6	0.44	5.2	**0.031** ↘	19.5	**< 0.001**	0.0	0.92	1.8	0.19	5.4	**0.028**
pCO_2	0.6	0.43	0.4	0.52	0.0	0.95	0.2	0.69	0.3	0.58	96.6	**< 0.001** ↘
Grazing × T	0.2	0.64	0.3	0.61	1.0	0.33	7.4	**0.011**	2.2	0.15	5.8	**0.022**
Grazing × pCO_2	0.1	0.77	0.3	0.62	0.3	0.58	2.5	0.12	8.6	**0.006**	1.9	0.18
pCO_2 × T	0.7	0.43	2.0	0.17	4.3	**0.048**	0.2	0.70	0.2	0.70	0.8	0.38
Grazing × T × pCO_2	0.5	0.48	0.0	0.84	1.9	0.18	0.0	0.84	1.9	0.18	1.1	0.31

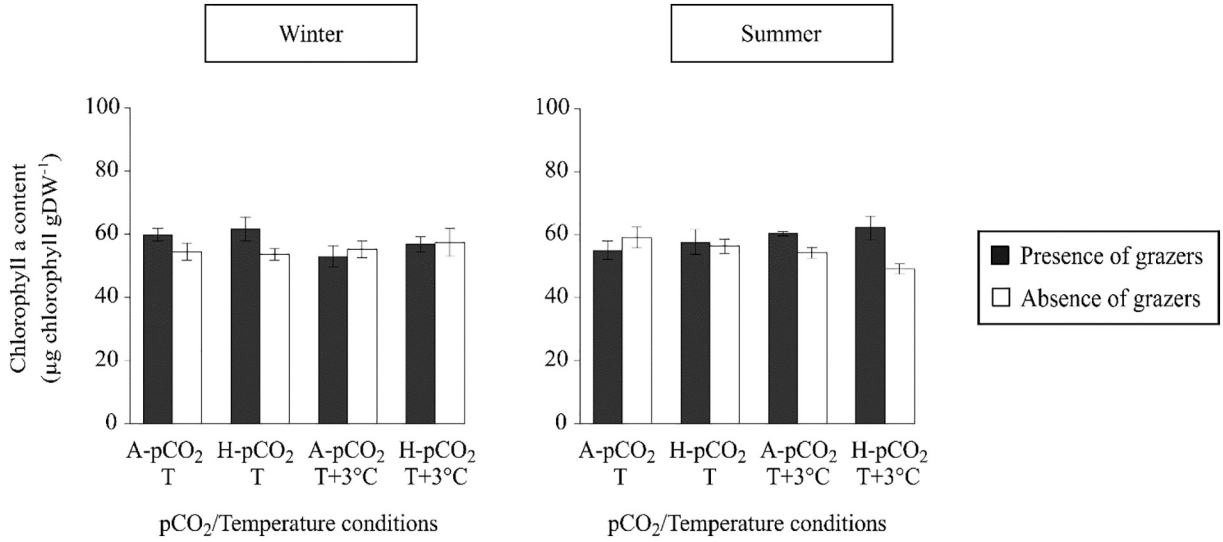

Fig. 2. Chlorophyll a content (mean ± SE, n = 5) of *L. corallioides* thalli maintained in the presence (black) and the absence of grazers (white) in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments. Results "with grazers" come from the work of Legrand et al. (2017).

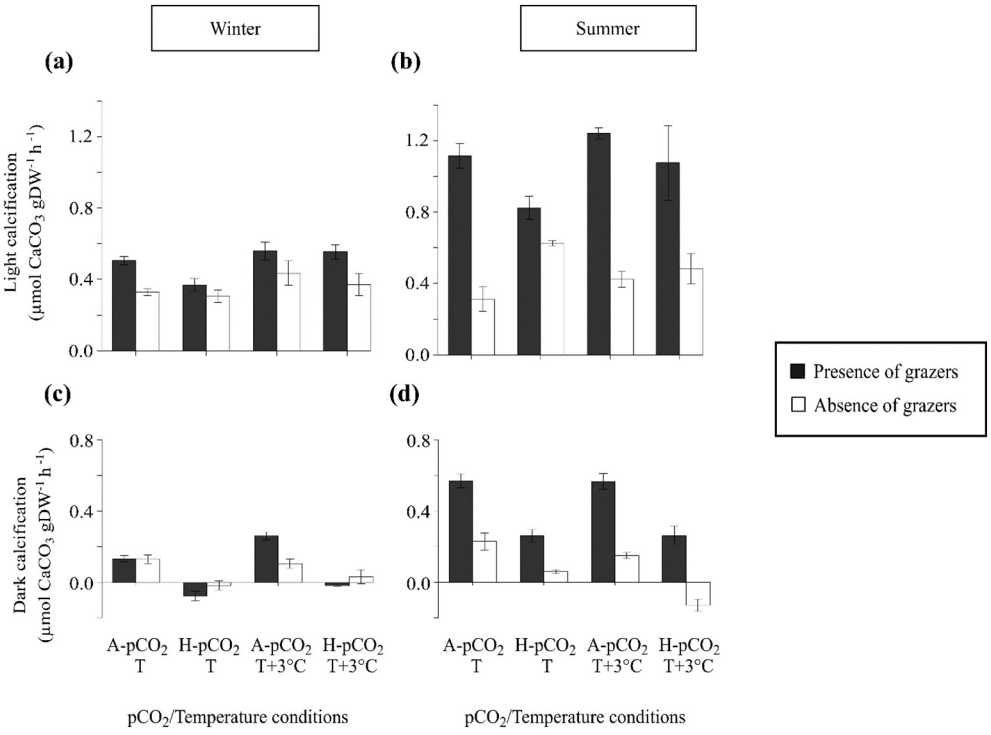

Fig. 3. Winter and summer light (a and b, respectively) and dark (c and d, respectively) calcification of *L. corallioides* in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments, after being maintained three months in presence (black) and absence (white) of grazers. Results are presented as means ± SE (n = 5). Results "with grazers" come from the work of Legrand et al. (2017).

surface (Wegeberg and Pueschel, 2002). This increases light availability for underlying photosynthetic cells and enhances primary production (Littler et al., 1995; Silliman and Zieman, 2001). The presence of compensatory mechanisms in *L. corallioides* may also increase the primary production in response to grazing (Lamberti and Moore, 1984; Wai and Williams, 2005). The present results also highlighted an increase in calcification in response to grazing, which may act as a structural protection for the algae (Steneck, 1983; Hay et al., 1994; Littler et al., 1995; Rahman and Halfar, 2014).

Several studies evidenced that moderated temperature rise usually enhanced photosynthesis, respiration and calcification in coralline algae (see review by Martin et al., 2013). The present data suggested that the relationship between temperature and physiological processes may be more complex, depending on the season and grazing condition. In the winter, increased temperature enhanced *L. corallioides* photosynthesis in the absence of grazers, while a decline was observed in their presence. On the other hand, calcification was positively affected by increased temperature, especially in the presence of grazers. In several coralline algae, photosynthesis involves carbon concentration mechanisms to convert bicarbonates (HCO_3^-) to CO_2 for Rubisco using carbonic anhydrase enzyme (Giordano et al., 2005; Hofmann et al., 2012). Hofmann et al. (2012) suggested that this enzyme may also be involved in the calcification process to convert CO_2 into HCO_3^- and then carbonates (CO_3^{2-}). It is likely that the increase in carbonic anhydrase activity with temperature may help algae to increase calcification in the winter and to maintain its structural protection in the presence of grazers. However, the allocation of carbonic anhydrase to the calcification process may be detrimental to photosynthesis, as these

two processes may thus be concurrent (Martin et al., 2013). This hypothesis would be consistent with the decline of photosynthesis observed under elevated temperature in the presence of grazers.

In the winter, the negative effect of pCO_2 rise on dark calcification was exacerbated in the presence of grazers. In the summer, the pCO_2 rise positively affected light calcification, while a negative effect was observed in the presence of grazers. In coralline algae, carbonate precipitation occurs in the cell walls, excepted for reproductive and epithelial cells, which are located just below the thallus surface (Irvine and Chamberlain, 1994). A part of epithelial cells was likely to be removed by grazers (Steneck, 1982), making calcified cells more exposed to the external environment. When grazing and pCO_2 increase are combined, calcified cells would thus be more exposed to dissolution, reducing light calcification rates. Moreover, calcification is generally considered as a structural defense in calcareous algae (Hay et al., 1994). Thus, ocean acidification is likely to affect their structural integrity increasing their vulnerability to grazing (Johnson and Carpenter, 2012; Ragazzola et al., 2012). In the urchin *Paracentrotus lividus*, Asnaghi et al. (2013) also evidenced an increase in coralline consumption under high pCO_2. This process may be essential in controlling the response of *P. lividus* to ocean acidification through a modulation of carbonate uptake (Asnaghi et al., 2013). In a context of ocean acidification, the presence of grazers may thus increase the sensitivity of *L. corallioides*. Despite this, the sensitivity of *L. corallioides* to ocean acidification is likely to be weakened when increased pCO_2 is combined with increased temperature in the presence of grazers, which differs from the conclusions of other studies (Anthony et al., 2008; Martin and Gattuso, 2009).

In conclusion, the present findings provide evidence that both season and grazing have a major impact on the physiology of *L. corallioides* and drive its response to ocean acidification and warming. In the winter, the ability of *L. corallioides* to enhance its calcification under elevated temperature may act as an important process to maintain the structural integrity of thalli in the presence of grazers. However, the metabolic cost of maintaining calcification to face grazing may be detrimental to other physiological processes, such as photosynthesis. In the summer, grazers potentially moderated epiphytic development, decreasing the light and nutrients competition induced by fast growing turf algae. Our results also evidenced that *L. corallioides* calcification was adversely affected when grazing was combined with increased pCO_2 in the summer. Through their feeding activity, grazers may alter the structural integrity of thalli and increase the sensitivity of coralline algae to ocean acidification. These results provide insights that grazers may have an important function in the response of coralline algae in the context of climate change. Field and laboratory experiments considering both seasonal variations and the response of species in multi-species assemblage are therefore critical to make ecologically relevant predictions of the effects of future environmental changes.

Aknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seares.2019.03.001.

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